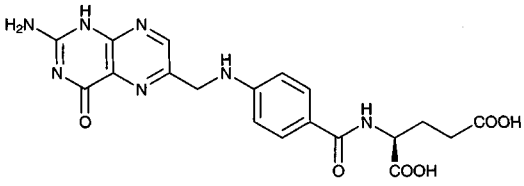
Detector: Chemiluminescence (418 nm cutoff filter) following post-column reaction. The column effluent mixed with 50 mM hydrogen peroxide in MeCN:dichloromethane 50:50 containing 0.5 mM triethylamine pumped at 0.1 mL/min and with 5 mM bis(2,4,6-trichlorophenyl) oxalate in dichloromethane pumped at 0.1 mL/min and the mixture flowed into the detector.

CHROMATOGRAM
Retention time: 7
Limit of detection: 5 nM

KEY WORDS
derivatization; normal phase

REFERENCE
Kwakman,P.J.M.; Koelewijn,H.; Kool,I.; Brinkman,U.A.T.; de Jong,G.J. Naphthalene- and anthracene-2,3-dialdehyde as precolumn labelling reagents for primary amines using reversed- and normal-phase liquid chromatography with peroxyoxalate chemiluminescence detection, *J.Chromatogr.*, **1990**, *511*, 155–166.

Folic acid



Molecular formula: $C_{19}H_{19}N_7O_6$
Molecular weight: 441.40
CAS Registry No.: 59-30-3
Merck Index: 4253

SAMPLE
Matrix: blood, formulations, urine
Sample preparation: Tablets. Powder tablets, dissolve in water, inject a 10 μL aliquot. Injections. Dilute with water, inject a 10 μL aliquot. Plasma, urine. Condition a Lichrolut RP-18 (Merck) SPE cartridge with 3 mL MeOH and 3 mL water. Mix 40 μL plasma or 100 μL urine with twice the volume of MeCN for 2 min, add 100 μL water, centrifuge at 3500 rpm for 15 min, evaporate the supernatant under nitrogen at 45° to remove the organic solvents, add slowly to the SPE cartridge, collect the eluate. Evaporate to dryness under a stream of nitrogen at 45°. Reconstitute the residue with 500 μL MeOH containing 4.2 μg/mL IS. Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Lichrosorb RP-18
Mobile phase: Gradient. A was MeOH. B was 50 mM ammonium acetate. A:B from 5:95 to 15:85 over 6 min, to 30:70 over 7 min, maintain at 30:70 over 7 min
Flow rate: 1
Injection volume: 10
Detector: UV 270

CHROMATOGRAM
Retention time: 9.99
Internal standard: xanthine (4.65)
Limit of detection: 3 ng

OTHER SUBSTANCES
Extracted: ascorbic acid, niacin, niacinamide, riboflavin, vitamin B12

KEY WORDS
plasma; SPE; tablets; injections

REFERENCE
Papadoyannis,I.N.; Tsioni,G.K.; Samanidou,V.F. Simultaneous determination of nine water and fat soluble vitamins after SPE separation and RP-HPLC analysis in pharmaceutical preparations and biological fluids, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 3203–3231.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85: 15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 3.583

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: bulk, formulations

Sample preparation: Bulk. Dilute 32 mg bulk drug in 100 mL IS solution, mix well. Remove a 4 mL aliquot and make it up to 100 mL with IS solution, inject an aliquot. Tablets. Powder tablets, weigh out amount equivalent to 300 μg folic acid, add 25 mL IS solution, flush tube with nitrogen, shake vigorously for 5 min, filter (Millipore type HA, 0.45 μm), flush filtrate container with nitrogen, inject an aliquot. Capsules. Weigh out amount of capsule filling equivalent to 300 μg folic acid, add 30 mL hexane, shake vigorously for 5 min, centrifuge at 1070 g for 15 min. Remove the hexane and dry the residue at 60°. Add 25 mL IS solution to the dry residue, flush tube with nitrogen, shake vigorously for 5 min, filter (Millipore type HA, 0.45 μm), flush filtrate container with nitrogen, inject an aliquot. (IS solution was 40 mg methyl paraben, 240 mL MeOH, 650 mL water, 12 mL 40% tetrabutylammonium hydroxide in water, 2.04 g KH_2PO_4, and 30 mL 100 mg/mL pentetic acid in 750 mM ammonium hydroxide made up to 1 L with water.)

HPLC VARIABLES

Column: 300 × 4 10 μm μBondapak C18

Mobile phase: MeOH:buffer 24:76 adjusted to pH 7.0 with phosphoric acid and ammonium hydroxide (Buffer was 7.5 mL 40% tetrabutylammonium hydroxide in water, 2.04 g KH_2PO_4, and 7 mL 1 M phosphoric acid in 760 mL water.)

Column temperature: 35

Flow rate: 1.5

Injection volume: 10

Detector: UV 280

CHROMATOGRAM

Retention time: 12

Internal standard: methyl paraben (17)

OTHER SUBSTANCES
Simultaneous: degradation products, p-aminobenzoic acid
Noninterfering: vitamins A, B6, B12, C, D, E, niacin, thiamine, pantothenic acid

KEY WORDS
protect from light; capsules; tablets

REFERENCE
Tafolla,W.H.; Sarapu,A.C.; Dukes,G.R. Rapid and specific high-pressure liquid chromatographic assay for folic acid in multivitamin-mineral pharmaceutical preparations, *J.Pharm.Sci.*, **1981**, *70*, 1273–1276.

SAMPLE
Matrix: formula, milk
Sample preparation: Mix 8.0 g powdered infant milk with 10 mL water to it. Mix the diluted powder or 10.5 g liquid infant milk with 1 g solid trichloroacetic acid, shake thoroughly with magnetic stirring for 10 min, centrifuge at 1250 g for 10 min, add 3 mL 4% trichloroacetic acid to the solid residue, mix thoroughly for 10 min, centrifuge, discard the solid phase. Combine the two acid extracts and make up to 10 mL with 4% trichloroacetic acid, filter (0.45 μm), inject an aliquot of the filtrate.

HPLC VARIABLES
Guard column: 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Column: 250 × 4.6 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Mobile phase: MeOH:buffer 15:85 (Buffer was 5 mM octanesulfonic acid and 0.5% triethylamine, pH 3.6.)
Flow rate: 1
Injection volume: 20
Detector: UV 261 for 6 min, UV 287 for 2 min, UV 290 for 5 min, UV 282 for 3 min, UV 268 for 3.5 min, UV 361 for 20.5 min, UV 246 for 20 min

CHROMATOGRAM
Retention time: 13
Limit of quantitation: ≤300 ng/mL

OTHER SUBSTANCES
Extracted: thiamine, riboflavin, pyridoxine, vitamin B12, niacinamide, pyridoxal, pyridoxamine

REFERENCE
Albalá-Hurtado,S.; Veciana-Nogués,M.; Izquierdo-Pulido,M.; Mariné-Font,A. Determination of water-soluble vitamins in infant milk by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *778*, 247–253.

SAMPLE
Matrix: formulations
Sample preparation: Dilute injections with water, inject a 50 μL aliquot. Dissolve tablets or capsule contents in water (warm if necessary), filter (0.5 μm PTFE), inject a 50 μL aliquot of the filtrate. (Dissolve tablets or other formulations containing proteinaceous material in water at 60°, add 5% trichloroacetic acid (to pH 4.4), filter, inject a 50 μL aliquot.)

HPLC VARIABLES
Guard column: pellicular Corasil
Column: 10 μm μBondapak C18
Mobile phase: Gradient. A was prepared by dissolving 1 g sodium dioctylsulfosuccinate in 170 mL MeOH, add 10 mL concentrated formic acid, make up to 800 mL with water, adjust pH to 2.5 with 1 M KOH, make up to 1 L. B was prepared by dissolving 1 g sodium dioctylsulfosuccinate in 450 mL MeOH, add 10 mL concentrated formic acid, make up to 800 mL with water, adjust pH to 4.6, make up to 1 L. A:B 100:0 for 19 min then 0:100 (step gradient) or A:B from 100:0 to 0:100 over 25 min (concave curve 9), maintain at 0:100 for 3 min, return to initial conditions over 2 min.
Flow rate: 1.5
Injection volume: 50
Detector: UV 280

CHROMATOGRAM
Retention time: 7 (step gradient), 8 (curve gradient)

OTHER SUBSTANCES
Simultaneous: niacin (UV 254), niacinamide (UV 254), pyridoxamine, thiamine (UV 254), riboflavin (UV 254), pyridoxine, ascorbic acid

KEY WORDS
injections; capsules; tablets

REFERENCE
Woollard,D.C. New ion-pair reagent for the high-performance liquid chromatographic separation of B-group vitamins in pharmaceuticals, *J.Chromatogr.*, **1984**, *301*, 470–476.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 100 × 4 3 μm Hypersil BDS-C18
Mobile phase: Gradient. MeCN:water adjusted to pH 2.1 from 0.3:99.7 to 25:75 over 11 min
Flow rate: 0.5
Detector: UV 220

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: biotin, caffeine, citric acid, niacinamide, niacin, pantothenic acid, riboflavin, saccharin, thiamine, pyridoxine, vitamin B12, ascorbic acid

KEY WORDS
tablets

REFERENCE
Hewlett Packard Leaflet 12-5091-7351 EUS, **1993**.

SAMPLE
Matrix: formulations
Sample preparation: Dilute liquid multivitamin formulations, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Lichrosorb RP-8
Mobile phase: Gradient. A was 10 mM KH_2PO_4 containing 5 mM sodium hexanesulfonate adjusted to pH 2.8 with phosphoric acid. B was MeOH. A:B from 90:10 to 71.8:28.2 over 4 min, maintain at 71.8:28.2 for 1.5 min, to 50:50 over 6.5 min, maintain at 50:50 for 5 min, return to initial conditions over 5 min
Flow rate: 1
Injection volume: 5
Detector: UV 272

CHROMATOGRAM
Retention time: 10.49
Internal standard: theobromine (8)
Limit of detection: 0.465 ng

OTHER SUBSTANCES
Simultaneous: niacin, niacinamide, thiamine, riboflavin, pyridoxine (UV 290)

KEY WORDS
liquid multivitamins; degas solutions with helium; protect from light

REFERENCE
Blanco,D.; Sánchez,L.A.; Gutiérrez,M.D. Determination of water soluble vitamins by liquid chromatography with ordinary and narrow-bore columns, *J.Liq.Chromatogr.*, **1994**, *17*, 1525–1539.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 30 × 4.6 10 μm octadecylsilica (Brownlee)
Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: Gradient. MeCN:33 mM pH 2.3 sodium phosphate buffer from 7.2:92.8 to 11.3:88.7 over 15 min. (At the end of each day flush system with water then 50-75 mL MeOH. Place a column of 37-53 μm silica (Whatman) between pump and injection valve.)
Flow rate: 1
Injection volume: 100
Detector: F ex 365 em >415 (filter) following post-column reaction. The column effluent mixed with the reagent pumped at 0.23 mL/min and the mixture flowed through a 5 m × 0.8 mm ID coil of PTFE tubing at 60° to the detector. (Reagent was 0.005% calcium hypochlorite (HTH dry chlorine, Olin, Overland KS) in 100 mM K_2HPO_4 containing 200 mM NaCl.)

CHROMATOGRAM
Retention time: 22

KEY WORDS
post-column reaction

REFERENCE
Gregory,J.F.,III; Sartain,D.B.; Day,B.P.F. Fluorometric determination of folacin in biological materials using high performance liquid chromatography, *J.Nutr.*, **1984**, *114*, 341–353.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:50 mM KH_2PO_4 90:10
Flow rate: 1
Detector: UV 210

CHROMATOGRAM
Retention time: 3.3

OTHER SUBSTANCES
Simultaneous: biotin, niacin, pantothenic acid, riboflavin, niacinamide

REFERENCE
MetaChem Catalog, **1995**, p. 21.

SAMPLE
Matrix: tissue

HPLC VARIABLES
Guard column: 20 × 2 pellicular C18
Column: Econosphere C18
Mobile phase: Gradient. A was 16 mM sodium phosphate and 4 mM tetrabutylammonium phosphate, pH 6.0. B was MeCN:16 mM sodium phosphate and 4 mM tetrabutylammonium phosphate, pH 6.0 20:80. A:B 100:0 for 5 min, to 40:60 over 30 min.
Flow rate: 1.5
Injection volume: 50
Detector: UV 290

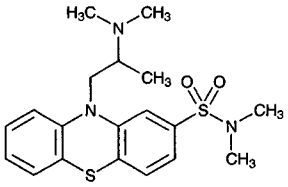
CHROMATOGRAM
Retention time: 21

OTHER SUBSTANCES
Extracted: other folates

KEY WORDS
rat; liver

REFERENCE
Rebello,T. Trace enrichment of biological folates on solid-phase adsorption cartridges and analysis by high-pressure liquid chromatography, *Anal.Biochem.*, **1987**, *166*, 55–64.

Fonazine



Molecular formula: $C_{19}H_{25}N_3O_2S_2$
Molecular weight: 391.56
CAS Registry No.: 7456-24-8, 7455-39-2 (methanesulfonate)
Merck Index: 4260

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.7

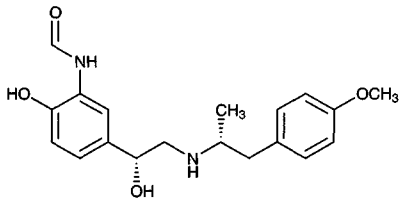
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pe-

cazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Formoterol



Molecular formula: $C_{19}H_{24}N_2O_4$
Molecular weight: 344.41
CAS Registry No.: 73573-87-2, 43229-80-7 (fumarate)
Merck Index: 4272

SAMPLE

Matrix: bulk
Sample preparation: Dissolve in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm Chiralcel OJ
Mobile phase: EtOH:heptane 30:70
Injection volume: 20
Detector: UV 365

CHROMATOGRAM

Retention time: k' 1.38 ((RR)-(+)), k' 2.00 ((SS)-(-))

KEY WORDS

chiral

REFERENCE

Francotte,E.R.; Richert,P. Applications of simulated moving-bed chromatography to the separation of the enantiomers of chiral drugs, *J.Chromatogr.A*, **1997**, *769*, 101–107.

SAMPLE

Matrix: bulk
Sample preparation: Dissolve 10 μmole compound (as free base or hydrochloride) in 500 μL MeCN, add 250 μL 5% sodium carbonate (for hydrochlorides only), add 500 μL 100 mM reagent in MeCN, vortex for 1 min, heat at 60° for 2 h, add 100 μmole L-proline, heat at 60° for 30 min. Remove a 100 μL aliquot and dilute it with mobile phase, neutralize with acetic acid, inject a 10 μL aliquot. Prepare the reagent ((R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate) as follows. Add 0.7 mL carbon disulfide to 6 mL (1R,2R)-(-)-1,2-diaminocyclohexane, 12 mL water, and 12 mL EtOH, heat the oil bath to 80°, add 2.8 mL carbon disulfide dropwise (making sure that the product does not start to precipitate), when addition is complete reflux for 1 h, acidify with 500 μL 5 M HCl, reflux for 12 h, cool, filter, wash the solid with a

little cold EtOH to give trans-4,5-tetramethyleneimidazolidine-2-thione as a white fluffy solid (mp 148-150°) (Tetrahedron 1993, 49, 4419). Stir 7.97 g 3,5-dinitrobenzoyl chloride in 30 mL dichloroethane at 50°, add a solution of 6 g trans-4,5-tetramethyleneimidazolidine-2-thione in 120 mL dichloroethane containing a catalytic amount of 4-(dimethylamino)pyridine over 15 min, reflux for 2 h, remove the crystals of (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate by filtration, evaporate the filtrate to dryness and dissolve the residue in 60 mL dichloroethane, reflux for 16 h to obtain more (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate (mp >250°, $[\alpha]_{546}$ = -133° (c = 1) in MeCN).

HPLC VARIABLES

Column: 125 × 4 5 μm Lichrospher 60 RP Select B
Mobile phase: MeCN:20 mM ammonium acetate 55:45
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: k' 2.52, k' 3.69 (enantiomers)

OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, atenolol, carazolol, carvedilol, methamphetamine, metipranolol, metoprolol, nifenanol, nitrilo atenolol, oxprenolol, pindolol, propranolol, xamoterol

KEY WORDS

derivatization; chiral

REFERENCE

Kleidernigg,O.P.; Posch,K.; Lindner,W. Synthesis and application of a new isothiocyanate as a chiral derivatizing agent for the indirect resolution of chiral amino alcohols and amines, *J.Chromatogr.A*, **1996**, *729*, 33–42.

SAMPLE

Matrix: urine
Sample preparation: Condition a 100 mg silica SPE cartridge with 3 mL MeOH and 3 mL ethyl acetate. Briefly vortex 1 mL urine with 100 μL 2.5 mg/mL IS in MeOH and 100 μL 250 mM pH 8.0 phosphate buffer, add 3 mL ethyl acetate, tumble at 25 rpm for 30 min, centrifuge at 4000 rpm for 10 min. Remove the organic layer and add it to the SPE cartridge, wash with 1 mL ethyl acetate, wash with 10 mL 5% isopropanol in water, centrifuge at 4000 rpm for 10 min. Elute with 3 mL MeOH, evaporate the eluate to dryness at 30° under a stream of nitrogen, reconstitute the residue with 100 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 4 Chiral AGP (Baker, Deventer, Netherlands)
Column: 100 × 4 Chiral AGP (Baker, Deventer, Netherlands)
Mobile phase: Isopropanol:50 mM pH 7.0 phosphate buffer 1.5:100, containing 1 mM KCl and a small quantity of EDTA (sic)
Flow rate: 0.9
Injection volume: 100
Detector: E, Waters EC 460, glassy carbon electrode +0.63 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 8.7 (R,R), 11.3 (S,S)
Internal standard: diastereomeric formoterol (Either R,S or S,R, prepared by preparative HPLC, details in paper) (15.5)
Limit of detection: 60 pmol/L (R,R), 75 pmol/L (S,S)

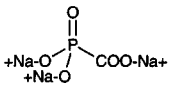
KEY WORDS

SPE; chiral; comparison with GC/MS; pharmacokinetics

REFERENCE

Butter,J.J.; van den Berg,B.T.J.; Portier,E.J.G.; Kaiser,G.; van Boxtel,C.J. Determination by HPLC with electrochemical detection of formoterol RR and SS enantiomers in urine, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 993–1005.

Foscarnet sodium



Molecular formula: CNa_3O_5P
Molecular weight: 191.95
CAS Registry No.: 63585-09-1
Merck Index: 4277

SAMPLE

Matrix: blood, CSF, urine

Sample preparation: Plasma. 100 μL Plasma + 900 μL 10 mM pyrophosphoric acid + 25 mg charcoal, after 30 s filter (Amicon MPS-1 with YMT membrane or Centricon 30 Microcencentrator) while centrifuging at 1000-2000 g for 15 min, dilute the ultrafiltrate 10-fold with 1 mM pyrophosphoric acid, inject a 20 μL aliquot. (For high concentrations of foscarnet charcoal may be omitted.) Urine. 100 μL Urine + 900 μL 10 mM pyrophosphoric acid + 25 mg charcoal, vortex for 30 s, filter (Millipore SJHVL04NS), dilute the filtrate 10-fold with 1 mM pH 5.8 pyrophosphoric acid, inject a 20 μL aliquot. CSF. Dilute CSF 100-fold with 1 mM pH 5.8 pyrophosphoric acid, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 3 μm UltroPac C18 (LKB)

Mobile phase: MeOH:pH 5.8 57 mM phosphate buffer 25:75 containing 1 mM tetrahexylammonium hydrogen sulfate and 0.2 mM pyrophosphoric acid

Flow rate: 1

Injection volume: 20

Detector: E, Environmental Sciences 5100 Coulochem, 5020 guard cell +0.75 V (placed after injector), 5010 analytical cell, cell 1 +0.75 V, cell 2 +0.90 V (monitored)

CHROMATOGRAM

Retention time: 4.66

Limit of quantitation: 30 μM (urine), 500 nM (plasma)

KEY WORDS

plasma; ultrafiltrate

REFERENCE

Pettersson,K.-J.; Nordgren,T.; Westerlund,D. Determination of phosphonoformate (foscarnet) in biological fluids by ion-pair reversed-phase liquid chromatography, *J.Chromatogr.*, **1989**, *488*, 447–455.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. Filter (Amicon Centricon 30) 1 mL plasma while centrifuging at 1500 g for 20 min, heat 200 μL of the ultrafiltrate in a boiling water bath for 20 min, cool, add 25-50 μL 671.8 μM hydrochlorothiazide in MeOH:water 50:50, vortex for 10 s, add 900 μL EtOH, mix for 20 s. Remove a 100 μL aliquot and dilute it to 1 mL with 1 mM pyrophosphoric acid, mix for 20 s, inject a 20 μL aliquot. Urine. 1 mL Urine + 9 mL 10 mM pyrophosphoric acid + 250 mg activated charcoal, vortex for 30 s. Filter (Amicon Centricon-30) 2 mL while centrifuging at 1500 g for 5 min. 200 μL Ultrafiltrate + 200 μL 20 mM NaOH + 400 μL EtOH, heat at 56° for 30 min, cool, add 671.8 μM hydrochlorothiazide in MeOH:water 50:50, mix for 10 s. Remove a 200 μL aliquot and dilute it 10-fold with 1 mM pyrophosphoric acid, mix for 20 s, inject a 20-40 μL aliquot.

HPLC VARIABLES

Column: 4 μm Nova-Pak C18

Mobile phase: MeOH:60 mM pH 5.8 buffer 30:70 containing 1 mM tetrahexylammonium hydrogen sulfate and 0.2 mM pyrophosphoric acid, pH adjusted to 5.8

Flow rate: 0.7

Injection volume: 20-40

Detector: E, ESA Coulochem Model 5100A, guard cell +0.99 V, analytical cell 1 +0.50 V, analytical cell 2 +0.95 V

CHROMATOGRAM
Retention time: 12.0
Internal standard: hydrochlorothiazide (18.9)
Limit of detection: 14 μM
Limit of quantitation: 33 μM

KEY WORDS
plasma; ultrafiltrate; pharmacokinetics

REFERENCE
Hassanzadeh,M.K.; Aweeka,F.T.; Wu,S.; Jacobson,M.A.; Gambertoglio,J.G. Determination of phosphonoformic acid in human plasma and urine by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1990**, *525*, 133–140.

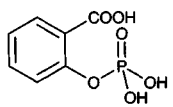
SAMPLE
Matrix: formulations
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeOH:5 mM sulfuric acid 5:95 containing 0.904 g/L tetrahexylammonium hydrogen sulfate
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

KEY WORDS
injections; saline

REFERENCE
Woods,K.; Steinmann,W.; Bruns,L.; Neels,J.T. Stability of foscarnet sodium in 0.9% sodium chloride solution, *Am.J.Hosp.Pharm.*, **1994**, *51*, 88–90.

Fosfosal



Molecular formula: $C_7H_7O_6P$
Molecular weight: 218.10
CAS Registry No.: 6064-83-1
Merck Index: 4281

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep Pak C18 SPE cartridge with 2 mL MeOH. Add 500 μL Plasma to the SPE cartridge, elute with 500 μL water. Collect all the eluate and centrifuge at 2500 rpm for 15 min, inject a 50 μL aliquot of the supernatant.

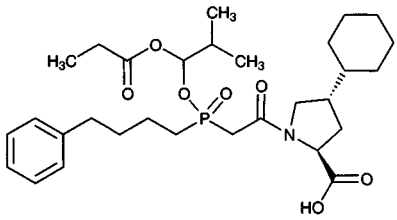
HPLC VARIABLES
Guard column: 10 μm μBondapak C18
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:5 mM tetrabutylammonium phosphate (PIC A) 30:70
Flow rate: 1
Injection volume: 50
Detector: UV 280

CHROMATOGRAM
Retention time: 5.5
Limit of detection: 1000 ng/mL

KEY WORDS
plasma; rat; dog; pharmacokinetics; SPE; also for humans (see Int.J.Clin.Pharmacol.ther.Toxicol. 1988; 26; 421)

REFERENCE
Ramis,J.; Mis,R.; Forn,J. Pharmacokinetics of fosfosal in rats and dogs, *Arzneimittelforschung*, **1989**, *39*, 74–77.

Fosinopril



Molecular formula: $C_{30}H_{46}NO_7P$
Molecular weight: 563.67
CAS Registry No.: 98048-97-6, 97825-24-6, 88889-14-9 (sodium salt)
Merck Index: 4282
Lednicer No.: 5 66

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:0.05% phosphate buffer 65:8:27, pH adjusted to 3.5 with phosphoric acid
Flow rate: 3
Detector: UV

KEY WORDS
perfusate buffer; rat

REFERENCE
Friedman,D.I.; Amidon,G.L. Passive and carrier-mediated intestinal absorption components of two angiotensin converting enzyme (ACE) inhibitor prodrugs in rats: enalapril and fosinopril, *Pharm.Res.*, **1989**, *6*, 1043–1047.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in mobile phase at a concentration of 100 μg/mL.

HPLC VARIABLES
Column: 150 × 3.9 5 μm RESOLVE (Waters)
Mobile phase: MeCN:water:orthophosphoric acid 4000:15:2
Column temperature: 32
Flow rate: 1
Injection volume: 20
Detector: UV 205

CHROMATOGRAM
Retention time: 5.13

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE
Kirschbaum,J.; Noroski,J.; Cosey,A.; Mayo,D.; Adamovics,J. High-performance liquid chromatography of the drug fosenopril, *J.Chromatogr.*, **1990**, *507*, 165–170.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 3.9 μBondapak phenyl
Mobile phase: MeOH:water:85% phosphoric acid 72:28:0.2
Column temperature: 30-40
Detector: UV 215-220

REFERENCE
Ranadive,S.A.; Chen,A.X.; Serajuddin,A.T. Relative lipophilicities and structural-pharmacological considerations of various angiotensin-converting enzyme (ACE) inhibitors, *Pharm.Res.*, **1992**, *9*, 1480–1486.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 4 10 μm alkylphenyl (Column Resolution Inc.)
Mobile phase: MeOH:0.2% phosphoric acid 72:28
Flow rate: 2
Injection volume: 50
Detector: UV 215

CHROMATOGRAM
Retention time: 10.5

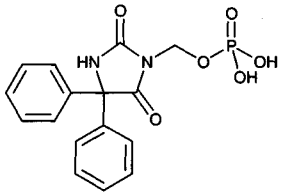
OTHER SUBSTANCES
Simultaneous: fosinoprilat, degradation products

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Lozano,R.; Warren,F.V.,Jr.; Perlman,S.; Joseph,J.M. Quantitative analysis of fosinopril sodium by capillary zone electrophoresis and liquid chromatography, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 139–148.

Fosphenytoin

Molecular formula: $C_{16}H_{15}N_2O_6P$
Molecular weight: 362.28
CAS Registry No.: 93390-81-9



SAMPLE
Matrix: formulations
Sample preparation: Dilute injection with MeCN:water 20:80, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 5 × 6 5 μm Guard-Pak Resolve C18
Column: 150 × 3.9 5 μm Resolve C18
Mobile phase: MeCN:water:85% phosphoric acid 25:75:0.09, containing 5 mM tetrabutylammonium sulfate
Column temperature: 30
Flow rate: 2
Injection volume: 20
Detector: UV 210

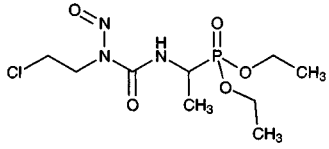
CHROMATOGRAM
Retention time: 9.1
Limit of quantitation: 15 μg/ml

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
injections; stability-indicating

REFERENCE
Fischer,J.H.; Cwik,M.J.; Luer,M.S.; Sibley,C.B.; Deyo,K.L. Stability of fosphenytoin sodium with intravenous solutions in glass bottles, polyvinyl chloride bags, and polypropylene syringes, *Ann.Pharmacother.*, **1997**, *31*, 553–559.

Fotemustine



Molecular formula: $C_9H_{19}ClN_3O_5P$
Molecular weight: 315.69
CAS Registry No.: 92118-27-9
Merck Index: 4285

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg CBA Bond Elut SPE cartridge with 1 mL MeOH and 1 mL water. Centrifuge blood at 5000 g for 2-3 min, freeze plasma in dry ice/hexane within 1 min. Thaw within 3 min by immersion in a 50° water bath. 1 mL Thawed plasma + 500 μL 2.5 μg/mL IS in 100 mM citric acid, vortex for 5 s, centrifuge for 5 min, add a 1 mL aliquot of the supernatant to the SPE cartridge, wash with 1 mL water, elute with 200 μL MeOH into a vial containing 50 μL 100 mM acetic acid, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 125 × 5 5 μm Spherisorb ODS
Mobile phase: MeCN:50 mM ammonium acetate 30:70 adjusted to pH 4.4 with glacial acetic acid
Flow rate: 1
Injection volume: 25
Detector: UV 230

CHROMATOGRAM
Retention time: 9.1
Internal standard: 1-methyl-3-isobutyl-8-vinyl-2,6-dioxopurine (S10338) (7.2)
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Extracted: lomustine, carmustine, metabolites

KEY WORDS
plasma; protect from light; pharmacokinetics; SPE; monkey; human

REFERENCE
Gordon,B.H.; Richards,R.P.; Hiley,M.P.; Gray,A.J.; Ings,R.M.; Campbell,D.B. A new method for the measurement of nitrosoureas in plasma: an h.p.l.c. procedure for the measurement of fotemustine kinetics, *Xenobiotica*, **1989**, *19*, 329–339.

SAMPLE
Matrix: blood

Sample preparation: Add fotemustine in ethanol PBS to plasma. Mix 100 μL plasma with 600 μL diethyl ether, rotate, centrifuge. Remove 400 μL of the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

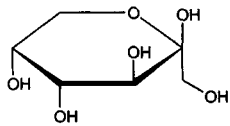
HPLC VARIABLES
Column: 150 mm long 4 μm Novapack
Mobile phase: EtOH:1% acetic acid (pH 3) 25:75
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7.6
Internal standard: carmustine (5.6)
Limit of detection: 1000 ng/mL

KEY WORDS
plasma; rat

REFERENCE
Meulemans,A.; Giroux,B.; Hannoun,P.; Henzel,D.; Bizzari,J.P.; Mohler,J. Permeability of two nitrosoureas, carmustine and fotemustine in rat cortex, *Chemotherapy*, **1989**, *35*, 313–319.

Fructose



Molecular formula: $C_6H_{12}O_6$
Molecular weight: 180.16
CAS Registry No.: 57-48-7
Merck Index: 4295

SAMPLE
Matrix: beverages, plants
Sample preparation: Beverages. Dilute 50-fold, filter (0.22 μm), inject an aliquot of the filtrate. Plants. Heat 1 g barley leaves and 10 mL EtOH:water 80:20 at 100° in a sealed tube for 15-30 min. Evaporate the liquid phase to dryness, reconstitute with water, pass through Analytichem trimethylaminopropyl and cyclohexyl SPE cartridges, inject an aliquot.

HPLC VARIABLES
Column: 300 × 6.5 Sugar-Pak I (Waters)
Mobile phase: water
Column temperature: 70
Flow rate: 0.4
Injection volume: 10
Detector: F ex 360 em 470 following post-column reaction. The effluent from the column passed through a 75 × 3.8 reactor containing Dowex 50 W × 2 sulfonic-acid type styrene divinylbenzene copolymer at 100° and mixed with 30 mM benzamidine in 1 M KOH pumped at 1 mL/min. This mixture flowed through a 530 μL reaction coil (Varian PCR-1) at 100° to the detector.

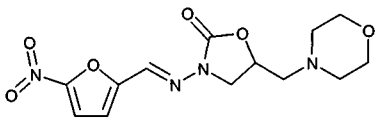
CHROMATOGRAM
Retention time: 22.77
Limit of detection: 60 pmole

OTHER SUBSTANCES
Extracted: dextrose, sucrose

KEY WORDS
barley; SPE; post-column reaction

REFERENCE
Coquet,A.; Haerdi,W.; Degli Agosti,R.; Veuthey,J.-L. Determination of sugars by liquid chromatography with post-column catalytic derivatization and fluorescence detection, *Chromatographia*, **1994**, *38*, 12–16.

Furaltadone



Molecular formula: $C_{13}H_{16}N_4O_6$
Molecular weight: 324.29
CAS Registry No.: 139-91-3
Merck Index: 4315
Lednicer No.: 1 229

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 347.4

CHROMATOGRAM
Retention time: 8.927

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: milk
Sample preparation: Mix 50 mL cow milk with 25 mL 20% trichloroacetic acid, let stand for 15 min. Filter the samples and wash with water. Adjust the pH to 4.5-5 with NaOH, make up to 100 mL with water. Take a 25 mL aliquot and add it to a Sep-Pak Plus C18 SPE cartridge, elute with 2.5 mL mobile phase, pass nitrogen through eluate for at least 2 min (to remove oxygen), inject an aliquot.

HPLC VARIABLES
Guard column: Symmetry C18
Column: 150 × 3.9 4 μm Nova Pak C18

Mobile phase: MeCN:100mM aqueous sodium perchlorate:glacial acetic acid 28:72:0.5
Flow rate: 1
Injection volume: 20
Detector: E, ESA Coulochem II, Model 5011 analytical cell, porous carbon electrode -600 V, Model 5021 conditioning cell

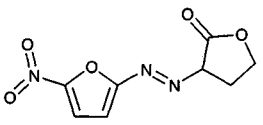
CHROMATOGRAM
Retention time: 2.5
Limit of detection: 4 ppb

OTHER SUBSTANCES
Extracted: furazolidone, nitrofurantoin

KEY WORDS
cow; SPE

REFERENCE
Galeano Diaz,T.; Guiberteau Cabanillas,A.; Acedo Valenzuela,M.I.; Correa,C.A.; Salinas,F. Determination of nitrofurantoin, furazolidone and furaltadone in milk by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.A*, **1997**, *764*, 243–248.

Furazolidone



Molecular formula: $C_8H_7N_3O_5$
Molecular weight: 225.16
CAS Registry No.: 67-45-8
Merck Index: 4320
Lednicer No.: 1 229

SAMPLE
Matrix: blood, eggs
Sample preparation: Dilute 1 mL serum or 0.5 mL egg yolk to 3 mL with water, mix, add to an Extrelut-3 SPE cartridge, elute with 14 mL ethyl acetate. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 500 μL mobile phase, centrifuge at 10000 rpm for 6 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5C18 HG (Wako)
Mobile phase: MeCN:water 40:60
Flow rate: 1
Injection volume: 50
Detector: UV 358

CHROMATOGRAM
Retention time: 3
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Noninterfering: chlortetracycline, oxolinic acid, oxytetracycline, sulfonamides, tylosin

KEY WORDS
pig; serum; chicken; SPE

REFERENCE
Yoshida,K.; Kondo,F. Liquid chromatographic determination of furazolidone in swine serum and avian egg, *J.AOAC Int.*, **1995**, *78*, 1126–1129.

SAMPLE
Matrix: blood, milk
Sample preparation: Extract 1 mL plasma or milk with 5 mL dichloromethane in acidic medium (pH 3), mix, centrifuge. Remove organic phase and evaporate it to dryness at 50° under a stream of nitrogen. Take up residue in MeCN and inject an aliquot. (Protect from light during extraction procedure.)

HPLC VARIABLES
Column: 75 × 4.6 Beckman XL 3 μm ODS
Mobile phase: MeCN:water 35:65
Flow rate: 1
Detector: UV 364

CHROMATOGRAM
Internal standard: furazolidone

OTHER SUBSTANCES
Simultaneous: nitrofurantoin

KEY WORDS
plasma; furazolidone is IS

REFERENCE
Pons,G.; Rey,E.; Richard,M.O.; Vauzelle,F.; Francoual,C.; Moran,C.; d'Athis,P.; Badoual,J.; Olive,G. Nitrofurantoin excretion in human milk, *Dev.Pharmacol.Ther.*, **1990**, *14*, 148–152.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 347.4

CHROMATOGRAM
Retention time: 12.24

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: eggs, tissue
Sample preparation: Add 30 mL MeCN to 10 g homogenized shelled eggs, liver, or muscle, blend at low speed for 2 min, centrifuge at 1000 g for 5 min, add 10 mL 10% NaCl solution and 50 mL dichloromethane to the supernatant, shake for a few min. Filter the lower organic layer through 5 g anhydrous sodium sulfate, evaporate the filtrate to dryness using a rotary vacuum evaporator at 45°, redissolve the residue in 1 mL MeCN:MeOH:20 mM pH 4.6 sodium acetate 10:50:40, inject an aliquot. (Protect from light. Wash the 1 mL of MeCN:MeOH:20 mM pH 4.6 sodium acetate 10:50:40 three times with 1 mL n-hexane before use.)

HPLC VARIABLES
Guard column: 10 × 4.6 μBondapak C18
Column: 150 × 4.6 5 μm Spherisorb ODS2 S5
Mobile phase: MeCN:20 mM pH 4.6 sodium acetate 21:79
Flow rate: 1
Injection volume: 50
Detector: UV 362

CHROMATOGRAM
Retention time: 8.2
Limit of detection: 2.5 ng/g

OTHER SUBSTANCES
Extracted: nitrofurazone, furaltadone

KEY WORDS
chicken; liver; muscle

REFERENCE
Draisci,R.; Giannetti,L.; Lucentini,L.; Palleschi,L.; Brambilla,G.; Serpe,L.; Gallo,P. Determination of nitrofuran residues in avian eggs by liquid chromatography-UV photodiode array detection and confirmation by liquid chromatography-ionspray mass spectrometry, *J.Chromatogr.A*, **1997**, *777*, 201–211.

SAMPLE
Matrix: eggs, tissue
Sample preparation: Add 30 mL MeCN to 10 g homogenized shelled eggs, liver, or muscle, blend at low speed for 2 min, centrifuge at 1000 g for 5 min, add 10 mL 10% NaCl solution and 50 mL dichloromethane to the supernatant, shake for a few min. Filter the lower organic layer through 5 g anhydrous sodium sulfate, evaporate the filtrate to dryness using a rotary vacuum evaporator at 45°, redissolve the residue in 1 mL MeCN:MeOH:20 mM pH 4.6 sodium acetate 10:50:40, inject an aliquot. (Protect from light. Wash the 1 mL of MeCN:MeOH:20 mM pH 4.6 sodium acetate 10:50:40 three times with 1 mL n-hexane before use.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil L C18-DB
Mobile phase: MeCN:water 50:50 containing 1 mM ammonium acetate and 0.025% acetic acid
Flow rate: 0.6
Injection volume: 20
Detector: MS, PESCIEX API I, ionspray interface 5500 V, OR 60 V, m/z 226, split the column effluent so that 0.03 mL/min enters the MS

CHROMATOGRAM
Retention time: 6.3
Limit of detection: 1.6 ng/g

KEY WORDS
chicken; liver; muscle

REFERENCE
Draisci,R.; Giannetti,L.; Lucentini,L.; Palleschi,L.; Brambilla,G.; Serpe,L.; Gallo,P. Determination of nitrofuran residues in avian eggs by liquid chromatography-UV photodiode array detection and confirmation by liquid chromatography-ionspray mass spectrometry, *J.Chromatogr.A*, **1997**, *777*, 201–211.

SAMPLE
Matrix: egs, milk, tissue
Sample preparation: Centrifuge milk at 2000 g for 10 min, freeze at -20° for 15 min, dilute a 10 mL aliquot with 10 mL saline, add 2 mL 1% sodium azide. Blend (Stomacher) 10 g homogenized tissue with 30 mL saline for 3 min, centrifuge at 2000 g, mix 20 mL of the supernatant with 2 mL 1% sodium azide. Dilute 10 mL homogenized egg with 10 mL saline, add 3 mL 10% sodium azide solution. Dialyze sample using a Cuprophan membrane (10000-15000 dalton cutoff) against water pumped at 0.36-1.44 mL/min for 3-9 min, pass the water through column A, flush the column with pure water for 8 min, backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. To increase sensitivity a number of sample batches can be dialyzed before the contents of column A are analyzed. (Caution! Sodium azide is carcinogenic, mutagenic, and highly toxic! Do not discharge to the sink!)

HPLC VARIABLES
Column: A 60 × 4.6 37-50 μm Bondapak C18/Corasil; B 250 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:100 mM pH 5 acetate buffer 20:80
Flow rate: 1
Detector: UV 365

CHROMATOGRAM
Retention time: 11
Limit of detection: 5 ng/mL (milk), 2 ng/g (tissue), 1 ng/g (eggs)

OTHER SUBSTANCES
Extracted: furaltadone, nitrofurantoin, nitrofurazone

KEY WORDS
protect from light; cow; muscle; dialysis

REFERENCE
Aerts,M.M.; Beek,W.M.; Brinkman,U.A. On-line combination of dialysis and column-switching liquid chromatography as a fully automated sample preparation technique for biological samples. Determination of nitrofuran residues in edible products, *J.Chromatogr.*, **1990**, *500*, 453–468.

SAMPLE
Matrix: feed
Sample preparation: Add 15 mL water to 5 g ground feed sample, let stand for 5 min, add 35 mL MeCN:MeOH 50:50, shake on a mechanical shaker for 30 min, filter through a glass fiber filter. Pass the filtrate through a column dry-packed with 4 g neutral alumina (Sigma), discard the first 4 mL of eluate, collect the next 8 mL eluate, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:10 mM pH 6.0 sodium acetate 20:80
Flow rate: 1.5
Injection volume: 20
Detector: UV 365

CHROMATOGRAM
Retention time: 7.2
Limit of detection: 1 μg/g

REFERENCE
McCracken,R.J.; Kennedy,D.G. Determination of furazolidone in animal feeds using liquid chromatography with UV and thermospray mass spectrometric detection, *J.Chromatogr.A*, **1997**, *771*, 349–354.

SAMPLE
Matrix: feed
Sample preparation: Grind feed to pass 20 mesh. 10 g Feed + 5 mL water, swirl, let stand for 5 min, add 50 mL DMF:water 95:5, shake vigorously for 15 s, let stand in the dark at room

temperature overnight, filter (paper). Add 15 mL of the filtrate to 5 g alumina (Alcoa F-20, 80-200 mesh) in a 300 × 10 glass column, discard first several mL of eluate, collect remaining eluate, inject an aliquot

HPLC VARIABLES
Guard column: 100 × 2 μBondapak C18/Corasil
Column: 300 × 4 μBondapak C18
Mobile phase: MeCN:1% acetic acid 20:80
Detector: UV 280, UV 365

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Extracted: carbadox, nitrofurazone

KEY WORDS
protect from light

REFERENCE
Thorpe,V.A. Sample preparation of carbadox, furazolidone, nitrofurazone, and ethopabate in medicated feeds for high pressure liquid chromatography, *J.Assoc.Off.Anal.Chem.*, **1980**, *63*, 981–984.

SAMPLE
Matrix: feed, formulations, milk
Sample preparation: Formulations. Dissolve formulation in DMF, filter, inject a 10 μL aliquot. Feeds. Stir 10 g finely ground feeds with 40 mL DMF for 30 min, centrifuge, filter, wash residues with DMF, dilute to 50 mL with DMF, inject a 10 μL aliquot. Milk. Lyophilize 200 mL milk, wash with 75 mL MeCN during 15 min. Extract residue with 15 mL DMF with stirring for 30 min, wash residue with a mixture of 25 mL MeCN+ 5 mL DMF. Combine all organic solutions and evaporate to dryness in vacuum. Treat residue with DMF, filter, dilute to 25 mL with DMF, filter before analysis, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 33 × 4.6 Perkin-Elmer Pecosphere 3x3 CR C18
Mobile phase: MeCN:100 mM pH 3.2 sodium acetate/acetic acid 10:90
Flow rate: 2
Injection volume: 10
Detector: UV 360

CHROMATOGRAM
Retention time: 1.77
Limit of detection: 4.2 ng

OTHER SUBSTANCES
Simultaneous: nitrofurantoin, furaltadone

REFERENCE
Galeano Díaz,T.; Lopez Martínez,L.; Martínez Galera,M.; Salinas,F. Rapid determination of nitrofurantoin, furazolidone and furaltadone in formulations, feed and milk by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1994**, *17*, 457–475.

SAMPLE
Matrix: feeds
Sample preparation: Add 15 mL water to 5 g ground feed sample, let stand for 5 min, add 35 mL MeCN:MeOH 50:50, shake on a mechanical shaker for 30 min, filter through a glass fiber filter. Pass the filtrate through a column dry-packed with 4 g neutral alumina (Sigma), discard the first 4 mL of eluate, collect the next 8 mL eluate, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: RP18 4-4 guard column (Merck)

Column: 125 × 4 5 μm LiChrospher RP18 (end-capped) (Merck)
Mobile phase: MeCN:100 mM ammonium acetate 35:65
Flow rate: 1
Injection volume: 50
Detector: MS, Hewlett-Packard Model HP5989A, positive ion mode, source 200°, thermospray stem 125°, multiplier voltage 2000 V, dynode voltage 8000 V, m/z 243

CHROMATOGRAM
Retention time: 2.75

REFERENCE
McCracken,R.J.; Kennedy,D.G. Determination of furazolidone in animal feeds using liquid chromatography with UV and thermospray mass spectrometric detection, *J.Chromatogr.A*, **1997**, *771*, 349–354.

SAMPLE
Matrix: milk
Sample preparation: Mix 50 mL cow milk with 25 mL 20% trichloroacetic acid, let stand for 15 min. Filter the samples and wash with water. Adjust the pH to 4.5-5 with NaOH, make up to 100 mL with water. Take a 25 mL aliquot and add it to a Sep-Pak Plus C18 SPE cartridge, elute with 2.5 mL mobile phase, pass nitrogen through eluate for at least 2 min (to remove oxygen), inject an aliquot.

HPLC VARIABLES
Guard column: Symmetry C18
Column: 150 × 3.9 4 μm Nova Pak C18
Mobile phase: MeCN:100mM aqueous sodium perchlorate:glacial acetic acid 28:72:0.5
Flow rate: 1
Injection volume: 20
Detector: E, ESA Coulochem II, Model 5011 analytical cell, porous carbon electrode -600 V, Model 5021 conditioning cell

CHROMATOGRAM
Retention time: 2.7
Limit of detection: 5-6 ppb

OTHER SUBSTANCES
Extracted: furaltadone, nitrofurantoin

KEY WORDS
cow; SPE

REFERENCE
Galeano Diaz,T.; Guiberteau Cabanillas,A.; Acedo Valenzuela,M.I.; Correa,C.A.; Salinas,F. Determination of nitrofurantoin, furazolidone and furaltadone in milk by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.A*, **1997**, *764*, 243–248.

SAMPLE
Matrix: shrimp
Sample preparation: Condition a 6 mL Bond Elut C18 SPE cartridge with 5 mL MeOH and 5 mL water. Homogenize (Tissuemizer) 5 g finely-chopped (by hand) shrimp and 20 mL MeCN at medium speed for 45 s, centrifuge at 3000 rpm for 5 min, decant the supernatant. Add 30 mL hexane saturated with MeCN to the supernatant, shake for 30 s, discard the hexane layer. Add 10 mL EtOH to the MeCN layer, evaporate under reduced pressure at 45° to 2-5 mL (until liquid looks milky), add 2 mL EtOH, continue evaporation until there is 2 mL of a thick liquid, add 2 mL EtOH, evaporate to dryness. Add 2 mL water to the residue, sonicate for 5 min, add to the SPE cartridge, wash with 4 mL water, elute at ≤3 mL/min with 5 mL MeCN. Evaporate the eluate to dryness under a stream of nitrogen at ≤45° (remove promptly when dry), reconstitute the residue in 1 mL mobile phase, filter (0.45 μm), inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4 5 μm ODS Hypersil C18

Column: 200 × 4.6 5 μm ODS Hypersil C18
Mobile phase: MeCN:1% aqueous acetic acid 25:75
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: UV 375

CHROMATOGRAM
Retention time: 5
Limit of quantitation: 4 ng/g

OTHER SUBSTANCES
Extracted: nitrofurazone

KEY WORDS
SPE

REFERENCE
Rupp,H.S.; Munns,R.K.; Long,A.R. Simultaneous determination of nitrofurazone and furazolidone in shrimp (*Penaeus vannamei*) muscle tissue by liquid chromatography with UV detection, *J.AOAC Int.*, **1993**, *76*, 1235–1239.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 200 × 4.6 5 μm Hypersil SAS or 150 × 4.6 5 μm Hypersil SAS
Mobile phase: MeCN:buffer 30:70 (Mobile phase was 340 mL 100 mM citric acid, 5 mL 100 mM trisodium citrate, and 5 mL 100 mM Na_2EDTA made up to 500 mL with MeCN.)
Flow rate: 2
Injection volume: 100
Detector: UV 370

CHROMATOGRAM
Retention time: 2.1

OTHER SUBSTANCES
Simultaneous: oxytetracycline, tetracycline, chlortetracycline

REFERENCE
Murray,J.; McGill,A.S.; Hardy,R. Development of a method for the determination of oxytetracycline in trout, *Food Addit.Contam.*, **1987**, *5*, 77–83.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH:water 30:70, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Resolve spherical C18 (Waters)
Mobile phase: MeOH:water 35:65
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 305

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Simultaneous: carbadox, nitrofurazone
Noninterfering: pyrantel

KEY WORDS
protect from light

REFERENCE
Roybal,J.E.; Munns,R.K.; Shimoda,W. Liquid chromatographic determination of carbadox residues in animal feed, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 653–657.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in chloroform at a concentration of 1 μg/mL, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Lichrospher RP-18
Mobile phase: MeCN:10 mM sodium acetate 20:80, pH 5
Column temperature: 30
Flow rate: 1.6
Injection volume: 20
Detector: UV 365

CHROMATOGRAM
Retention time: 13.0
Limit of detection: 60 ng/mL

OTHER SUBSTANCES
Simultaneous: degradation products, carbadox, nitrofurazone, nitrofurantoin, furaltadone

REFERENCE
Kaniou,I.; Zachariadis,G.; Kalligas,G.; Tsoukali,H.; Stratis,J. Separation and determination of carbadox, nitrofurazone, nitrofurantoin, furazolidone, and furaltadone in their mixtures by thin layer and high performance liquid chromatography, *J.Liq.Chromatogr.*, **1994**, *17*, 1385–1398.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Waring blender) 10 g muscle, liver, or kidney in 100 mL EtOH for 5 min, let stand for 5 min, filter through 10 g Celite 545 on top of a sintered glass filter, rinse blender with 100 mL EtOH and filter rinse. Add 25 mL 3.6% aqueous metaphosphoric acid to the combined filtrates, evaporate to 25 mL under reduced pressure at 45°. Remove residue, rinse out flask with 5 mL hexane and 3 mL water, combine, centrifuge at 0° at 27000 g for 30 min, discard hexane, rinse surface with 5 mL hexane, discard hexane. Remove aqueous layer, rinse out tube twice with 3 mL portions of water, combine, add 10 mL 1 M KH_2PO_4, make up to 100 mL with water, extract three times for 5 min with 50 mL ethyl acetate. Combine the extracts and dry them over 15 g anhydrous sodium sulfate, filter through glass wool, evaporate to dryness under reduced pressure at 45°. Take up residue in 3 mL ethyl acetate and add to alumina column, rinse flask with 2 mL ethyl acetate and add rinse to column. Elute with 20 mL EtOH:MeOH:ethyl acetate 10:10:80 and combine all the eluate. Evaporate to dryness under reduced pressure at 45°, reconstitute in 500 μL mobile phase, inject a 100 μL aliquot. (Prepare alumina column by slurrying 1 g aluminum oxide (Baker) in 20 mL ethyl acetate and adding to a 200 × 6 glass chromatographic column.)

HPLC VARIABLES
Guard column: Brownlee 10 μm RP-GU MPLC C-8
Column: 250 × 4.6 Brownlee RP-10A C-8
Mobile phase: MeCN:EtOH:10 mM ammonium acetate 25:5:70, pH 6.8
Flow rate: 1
Injection volume: 100
Detector: UV 350

CHROMATOGRAM
Retention time: 8.7
Limit of detection: 2 ng
Limit of quantitation: 10 ng

OTHER SUBSTANCES
Extracted: quinoxaline-2-carboxylic acid, carbadox, nitrofurazone, desoxycarbadox

KEY WORDS
protect from light; pig; muscle; liver; kidney

REFERENCE
MacIntosh,A.I.; Neville,G.A. Liquid chromatographic determination of carbadox, desoxycarbadox, and nitrofurazones in pork tissues, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 958–962.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 500 mg Bond Elut C18 SPE cartridge with 5 mL MeOH and 10 mL water. Homogenize 5 g tissue with 100 mL MeOH:0.2% metaphosphoric acid 40:60 for 2 min, filter through a 1 mm layer of Hyflo Super-Cel. Evaporate the filtrate under reduced pressure at 40° to 10 mL, add the residue to the SPE cartridge, wash with 10 mL water, elute with 10 mL MeOH. Evaporate the eluate to dryness under reduced pressure, take up the residue in 1 mL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 Newguard RP-8
Column: 150 × 4.6 5 μm Inertsil ODS
Mobile phase: MeCN:5 mM oxalic acid 45:55
Flow rate: 0.5
Injection volume: 10
Detector: UV 265

CHROMATOGRAM
Retention time: 7
Limit of detection: 50 ng/g

OTHER SUBSTANCES
Extracted: sulfamonomethoxine, sulfadimethoxine, sulfisozole, nalidixic acid, oxolinic acid, piromidic acid, sodium nifurstyrenate

KEY WORDS
fish; SPE

REFERENCE
Horie,M.; Saito,K.; Hoshino,Y.; Nose,N.; Nakazawa,H.; Yamane,Y. Simultaneous determination of residual synthetic antibacterials in fish by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *538*, 484–491.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax TP 18/2) 3 g ground muscle + 200 μL 1 μg/mL nitrofurazone in water + 6.8 mL MeCN for 6 s, centrifuge at 5000 rpm for 5 min. Remove 6.5 mL of the supernatant and add it to 2 mL 5 M NaCl, shake vigorously for 10 s, centrifuge at 3000 rpm for 2 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 43°, reconstitute the residue in 250 μL MeCN:buffer 20:80, add 1 mL hexane, mix (Whirlimixer), centrifuge for 4 min, discard the hexane layer, filter (Costar Spin-X 0.22 μm cellulose acetate) while centrifuging at 5600 g for 4 min, inject a 20 μL aliquot of the filtrate. (Buffer was 20 mM sodium 1-heptanesulfonate and 10 mM Na_2HPO_4, pH adjusted to 6.0 with phosphoric acid.)

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelcosil LC-ABZ
Column: 250 × 4.6 5 μm Supelcosil LC-ABZ
Mobile phase: MeCN:buffer 25:75 (Buffer was 4.45 g sodium 1-heptanesulfonate and 9.5 g $Na_3PO_4.12H_2O$ in 750 mL water, adjust pH to 2.5 with 5 M phosphoric acid, make up to 1 L with water.)

Flow rate: 1
Injection volume: 20
Detector: UV 365

CHROMATOGRAM
Retention time: 7.5
Internal standard: nitrofurazone (5.5)
Limit of quantitation: 3 ng/g

KEY WORDS
cow; muscle

REFERENCE
Hormazábal,V.; Yndestad,M. Simple and rapid method of analysis for furazolidone in meat tissues by high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1995**, *18*, 1871–1877.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 500 mg Bond-Elut NH_2 SPE cartridge with 20 mL chloroform: MeOH 70:30 and 10 mL hexane. Homogenize 2 g pulverized tissue with 40 mL MeOH:buffer 30:70 for 1 min, centrifuge at 2000 rpm for 15 min, evaporate to about 15 mL under reduced pressure at 40°, add 25 mL dichloromethane, shake gently for 1 min, centrifuge at 1500 rpm for 10 min. Remove the lower organic layer and evaporate it to near dryness under reduced pressure at 30°. Resuspend the residue in 2 mL dichloromethane and 6 mL hexane, add to the SPE cartridge, wash with 5 mL hexane:dichloromethane 50:50, elute with 5 mL chloroform: MeOH 70:30. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot. (Buffer was 23 g Na_2HPO_4 and 14.2 g citric acid in 1 L water, pH 3.6.)

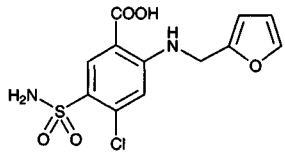
HPLC VARIABLES
Guard column: RP18 4-4 (Merck)
Column: 125 × 4 5 μm Lichrospher RP18 (end-capped)
Mobile phase: MeCN:100 mM ammonium acetate 25:75
Flow rate: 1
Injection volume: 50
Detector: MS, Vestec LC-MS Model 201A, thermospray, positive-ion mode, filament-assisted ionization, electron-beam current 300 μA, block 250°, tip 245°, lens 140°, vaporizer probe 180°, electron multiplier 2000 V, SIM m/z 243 $(M + NH_4)^+$

CHROMATOGRAM
Retention time: 3.6
Limit of detection: 1 ng/g

KEY WORDS
protect from light; pig; liver; kidney; diaphragm; muscle; pharmacokinetics; SPE

REFERENCE
McCracken,R.J.; Blanchflower,W.J.; Rowan,C.; McCoy,M.A.; Kennedy,D.G. Determination of furazolidone in porcine tissue using thermospray liquid chromatography-mass spectrometry and a study of the pharmacokinetics and stability of its residues, *Analyst*, **1995**, *120*, 2347–2351.

Furosemide



Molecular formula: $C_{12}H_{11}ClN_2O_5S$
Molecular weight: 330.75
CAS Registry No.: 54-31-9
Merck Index: 4331
Lednicer No.: 1 134; 2 87

SAMPLE
Matrix: blood
Sample preparation: Filter 900 or 1350 μL serum through a Tosoh Plastic filter (Japan) while centrifuging at 3000 or 5000 g for 15 min. Inject an aliquot.

HPLC VARIABLES
Column: Superspher 100RP-18e
Mobile phase: MeCN:MeOH:water 9:4:491
Flow rate: 1
Detector: UV 285

KEY WORDS
serum; pharmacokinetics; ultrafiltrate

REFERENCE
Takamura,N.; Maruyama,T.; Otagiri,M. Effects of uremic toxins and fatty acids on serum protein binding of furosemide: possible mechanism of the binding defect in uremia, *Clin.Chem.*, **1997**, *43*, 2274–2280.

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL 10 μg/mL nitrazepam in MeOH to 1 mL plasma, vortex, add 500 μL 200 mM pH 9.0 glycine buffer and 5 mL ethyl acetate, extract. Centrifuge at 700 g for 10 min, evaporate 4 mL of the organic phase to dryness under nitrogen at 60°, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:buffer 69:31 (Mobile phase was 690 mL MeCN, 310 mL water, and 9 mL glacial acetic acid, adjusted to pH 5.0 with 5 M NaOH.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 5.2
Internal standard: nitrazepam (10.5)
Limit of detection: 10 ng/mL
Limit of quantitation: 30 ng/mL

KEY WORDS
pharmacokinetics; plasma

REFERENCE
Jankowski,A.; Skorek-Jankowska,A.; Lamparczyk,H. Determination and pharmacokinetics of a furosemide-amiloride drug combination, *J.Chromatogr.B*, **1997**, *693*, 383–391.

SAMPLE
Matrix: blood
Sample preparation: Filter 170 μL serum (UFC30HV00 membrane filter, 0.45 μm, Millipore) and inject a 100 μL aliquot of the filtrate onto column A, elute to waste with mobile phase A,

after 4.6 min elute the contents of column A onto column B with mobile phase A:B 82:18, after 7 min elute column B with mobile phase C, monitor the effluent from column B. (After 7 min remove column A from the circuit and wash it with mobile phase A:B 40:60 for 4 min then equilibrate it with mobile phase A for 5 min. After 11 min re-equilibrate column B with mobile phase A for 6 min.)

HPLC VARIABLES
Column: A 10 μm Guard-pak μBondapak C18 (Waters); B 150 × 4.6 5 μm YMC Pack ODS A-type (Yamamura Chemicals, Japan)
Mobile phase: A 20 mM pH 7 phosphate buffer; B MeCN; C MeCN:20 mM pH 7 phosphate buffer 35:65 containing 15 mM tetra-*n*-butylammonium
Column temperature: 40 (column B)
Flow rate: 2
Injection volume: 100
Detector: UV 271

CHROMATOGRAM
Retention time: 14.8
Limit of quantitation: 5 ng/mL

KEY WORDS
column-switching; serum

REFERENCE
Okuda,T.; Yamashita,K.; Motohashi,M. High-performance liquid chromatography using on-line solid-phase extraction: determination of furosemide in human serum, *J.Chromatogr.B*, **1996**, *682*, 343–348.

SAMPLE
Matrix: blood, perilymph, tissue
Sample preparation: Add 5 μL 1100 μg/mL p-nitrophenol to 50 μL serum. Homogenize tissue in MeOH:water 80:20, centrifuge at 4° at 500 g for 5 min, remove 100 μL of the resulting supernatant and add internal standard. Pool perilymph to yield a 3 μL perilymph sample. Acidify all samples with 5 μL 2 M phosphoric acid (except 3 μL for perilymph), extract with 200 μL ethyl acetate, dry under nitrogen, reconstitute in 30 μL mobile phase, inject a 15 μL aliquot. (Perform all extractions in the dark.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm C18 (Beckmann)
Mobile phase: MeOH:10 mM pH 5.5 KH_2PO_4 buffer 37:63
Flow rate: 1.5
Injection volume: 15
Detector: UV 235

CHROMATOGRAM
Retention time: 5.64
Internal standard: p-nitrophenol (8.30)
Limit of detection: <100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, furosemide glucuronide

KEY WORDS
rat; serum; kidney; liver

REFERENCE
Mills,C.D.; Whitworth,C.; Ryback,L.P.; Henley,C.M. Quantification of furosemide from serum and tissues using high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *701*, 65–70.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Mix 500 μL plasma with 30 μL 10 mg/mL warfarin in MeOH, 50 μL 6 M hydrochloric acid and 3 mL diethyl ether, vortex for 30 s, centrifuge at 2000 g for 10

min, evaporate ether layer in a 45° water bath under a stream of nitrogen. Reconstitute the residue in 100 μL MeOH and inject a 20 μL aliquot. Urine. Mix 200 μL urine with 20 μL 6 M hydrochloric acid, 40μL 10 mg/mL warfarin in MeOH and 8 mL diethyl ether, vortex for 30 s, evaporate to dryness in a 45° water bath under a stream of nitrogen. Reconstitute the residue in 100 μL mobile phase and inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 10 μm μBondapak C18 (plasma), 150 × 3.9 5 μm Resolve Spherical C18 (urine)
Mobile phase: MeCN:buffer 38:62 (Buffer was 10 mM potassium dihydrogen phosphate adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 1.5
Injection volume: 20
Detector: F ex 229 em 389

CHROMATOGRAM

Retention time: 3
Internal standard: warfarin (9)
Limit of detection: 2-3 ng/ mL
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES

Simultaneous: quinidine, sulfamethoxazole
Noninterfering: metabolites, carbamazepine, cimetidine, diazepam, disopyramide, fluvoxamine, meclofenamate, metoclopramide, phenobarbital, phenylbutazone, phenytoin, ranitidine, theophylline, trimethoprim

KEY WORDS

pharmacokinetics; plasma

REFERENCE

Abou-Auda,H.S.; Al-Yamani,M.J.; Morad,A.M.; Bawazir,S.A.; Khan,S.Z.; al-Khamis,K.I. High-performance liquid chromatographic determination of furosemide in plasma and urine and its use in bioavailability studies, *J.Chromatogr.B*, **1998**, *710*, 121–128.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. Mix 400 μL plasma with 700 μL MeOH:acetone 60:40, centrifuge at 3000 rpm for 10 min. Add 400 μL of the supernatant to 50 μL 50 ng/mL IS, filter (0.5 μm), inject an aliquot. Urine. Mix 1 mL urine with 700 μL pH 4.8 acetate buffer and 300 μL 100 ng/mL IS, filter (0.5 μm), inject an aliquot.

HPLC VARIABLES

Guard column: 10 × 4 5 μm Shim-pack G-ODS
Column: 150 × 6 5 μm Shim-pack CLC-ODS
Mobile phase: Gradient. A was MeCN:water 20:80 containing 0.3% acetic acid. B was MeCN:water 80:20 containing 0.3% acetic acid. A:B 88:12 for 5 min, 60:40 for 5 min, 50:50 for 10 min, 88:12 for 10 min (step gradient).
Column temperature: 40
Flow rate: 1
Injection volume: 10-50
Detector: F ex 345 em 415

CHROMATOGRAM

Retention time: 13.8
Internal standard: bumetanide (17.4)
Limit of detection: 5 ng/mL (urine), 1 ng/mL (plasma)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Yagi,N.; Kiuchi,T.; Satoh,H.; Terashima,Y.; Kenmotsu,H.; Sekikawa,H.; Takada,M. Bioavailability and diuretic effect of furosemide following administration of tablets and retarded capsules to human subjects, *Biol.Pharm.Bull.*, **1996**, *19*, 616–622.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 234.6

CHROMATOGRAM
Retention time: 15.17

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4.6 5 μm C18
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 1.2
Injection volume: 20
Detector: UV 228

CHROMATOGRAM
Retention time: 4.52

OTHER SUBSTANCES
Simultaneous: granisetron (UV 300)

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE
Matrix: formulations, urine
Sample preparation: Tablets. Pulverize tablets, add MeOH, shake for 20 min, filter, wash solid with MeOH, dilute filtrate with mobile phase, inject a 20 μL aliquot. Urine. 2 mL Urine + 2 mL 1 M pH 3.25 KH_2PO_4 + 4 mL ethyl acetate, vortex for 20 min, centrifuge at 734 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 2 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: μBondapak C18
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water 40:60 containing 5 mM KH_2PO_4/K_2HPO_4, pH adjusted to 4.25
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: E, EG&G Princeton Applied Research PAR Model 400, glassy carbon working electrode +1200 mV, Ag/AgCl reference electrode (At the end of each day clean electrode with mobile phase of MeOH at 1.5 mL/min, -800 mV for 2 min then +1600 mV for 15 min.)

CHROMATOGRAM
Retention time: 7.7
Limit of quantitation: 15 ng/mL

OTHER SUBSTANCES
Extracted: piretanide

KEY WORDS
tablets; pharmacokinetics

REFERENCE
Barroso,M.B.; Jimenez,R.M.; Alonso,R.M.; Ortiz,E. Determination of piretanide and furosemide in pharmaceuticals and human urine by high-performance liquid chromatography with amperometric detection, *J.Chromatogr.B*, **1996**, *675*, 303–312.

SAMPLE
Matrix: formulations, urine
Sample preparation: Tablets. Pulverize tablets, add MeOH, shake for 30 min, sonicate for 5 min, filter (Albet 242 paper), wash solid with MeOH, make up filtrate to 50 mL with MeOH, inject a 20 μL aliquot. Urine. Adjust pH of 2 mL urine to 10.0 with 2 M KOH, add 1.5 mg NaCl, add 4 mL ethyl acetate, shake for 10 min, centrifuge at 2500 rpm for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 2 mL mobile phase, sonicate, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: μBondapak C18
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water 30:70 containing 5 mM KH_2PO_4/K_2HPO_4, pH adjusted to 5.5
Flow rate: 1
Injection volume: 20
Detector: E, EG&G Princeton Applied Research PAR Model 400, glassy carbon working electrode +1300 mV, Ag/AgCl reference electrode (At the end of each day clean electrode with mobile phase of MeOH at 1.5 mL/min, -800 mV for 2 min then +1600 mV for 5 min.)

CHROMATOGRAM
Retention time: 6.70
Limit of detection: 15 ng/mL

OTHER SUBSTANCES
Extracted: triamterene

KEY WORDS
tablets; pharmacokinetics

REFERENCE
Barroso,M.B.; Alonso,R.M.; Jiménez,R.M. Simultaneous determination of the diuretics triamterene and furosemide in pharmaceutical formulations and urine by HPLC-EC, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 231–246.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: THF:glacial acetic acid:water 40:1:59
Flow rate: 1
Detector: UV 276

REFERENCE
Walter,E.; Janich,S.; Roessler,B.J.; Hilfinger,J.M.; Amidon,G.L. HT29-MTX/Caco-2 cocultures as an in vitro model for the intestinal epithelium: In vitro-in vivo correlation with permeability data from rats and humans, *J.Pharm.Sci.*, **1996**, *85*, 1070–1076.

SAMPLE
Matrix: urine
Sample preparation: Inject 5 μL urine onto column A and elute to waste with mobile phase A, after 1 min backflush the contents of column A onto column B with mobile phase B. Monitor the effluent from column B.

HPLC VARIABLES
Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 125 × 4 5 μm LiChrospher 100 RP 18
Mobile phase: A 50 mM pH 3 phosphate buffer; B MeCN:50 mM pH 3 phosphate buffer 60:40 (Prepare buffer as follows. Dissolve 3.45 g NaH_2PO_4 monohydrate in 500 mL water containing 750 μL propylamine hydrochloride, adjust to pH 3 with concentrated phosphoric acid.)
Flow rate: 1
Injection volume: 5
Detector: UV 254, F ex 233 em 389

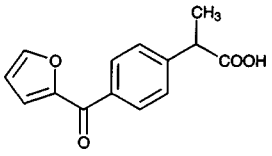
CHROMATOGRAM
Retention time: 7.7
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: amiloride, bumetanide, triamterene

KEY WORDS
column-switching

REFERENCE
Campins-Falcó,P.; Herráez-Hernández,R.; Pastor-Navarro,M.D. Analysis of diuretics in urine by column-switching chromatography and fluorescence detection, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 1867–1885.

Furprofen



Molecular formula: $C_{14}H_{12}O_4$
Molecular weight: 244.25
CAS Registry No.: 66318-17-0

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL plasma with 20 µL 100 µg/mL IS in 20 mM NaOH. Add 500 µL 50 mM pH 7.0 phosphate buffer, vortex for 1 min, add 2 mL dichloromethane, vortex for 1 min, shake for 5 min. Centrifuge at 100 g for 10 min, separate the organic layer, repeat the extraction procedure twice, evaporate the combined organic lowers to dryness under reduced pressure. Reconstitute with 200 µL 20 mM NaOH, inject a 20 µL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 10 µm Vydac AXGU
Column: 250 × 4.6 5 µm Supelcosil LC-SAX
Mobile phase: MeCN:50 mM pH 7.0 phosphate buffer 10:90
Flow rate: 1.5
Injection volume: 20
Detector: UV 280

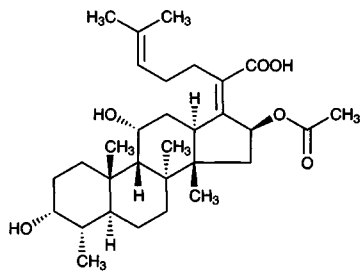
CHROMATOGRAM
Retention time: 4.9
Internal standard: fenbufen (3.5)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: rufloxacin

KEY WORDS
SPE; plasma

REFERENCE
Carlucci,G.; Mazzeo,P. Simultaneous determination of furprofen and rufloxacin in human plasma by high-performance liquid chromatography, *J.Chromatogr.Sci.*, **1996**, *34*, 182–184.

Fusidic acid



Molecular formula: $C_{31}H_{48}O_6$
Molecular weight: 516.72
CAS Registry No.: 6990-06-3, 751-94-0 (sodium salt)
Merck Index: 4340

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation.

Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 24.86

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: formulations
Sample preparation: Weigh out ointment containing 2.14 mg sodium fusidate, take up in 100 mL MeOH:water 20:80. Remove a 200 μL aliquot and add it to 150 μL 20 mM tetrahexylammonium bromide in 100 mM pH 7.0 phosphate buffer and 100 μL 4.2 mg/mL 2-bromoacetyl-6-methoxynaphthalene in acetone, mix, let stand at room temperature for 5 min, add 150 μL 8.9 μg/mL IS in MeCN, sonicate at room temperature for 1 min, inject a 50 μL aliquot. (Prepare 2-bromoacetyl-6-methoxynaphthalene by stirring equimolar amounts of 2-acetyl-6-methoxynaphthalene (Janssen Chimica, Belgium) and phenyltrimethylammonium tribromide in THF at room temperature for 3 h (Phosphorus and Sulfur 1985, 25, 357), purify by column chromatography on silica gel with chloroform:petroleum ether 50:50 (mp 109-112°) (Chromatographia 1992, 33, 13).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:MeOH:water 51:34:15
Column temperature: 35
Flow rate: 1.6
Injection volume: 50
Detector: F ex 300 em 460

CHROMATOGRAM

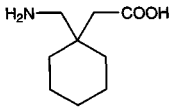
Retention time: 10
Internal standard: nonanoic acid naphthacyl ester (Prepare as follows. Dissolve 2 mmoles nonanoic acid and 1 mmole 2-bromoacetyl-6-methoxynaphthalene in 10 mL anhydrous MeCN, add 500 μL triethylamine, heat to 60° for 30 min, cool, add 30 mL water, extract three times with 10 mL portions of ether. Combine the extracts and wash them with 5% sodium bicarbonate solution and with three 10 mL portions of water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from MeOH/water (mp 66-8°) (Chromatographia 1992, 33, 13).) (5.5)
Limit of detection: 1 pmole

KEY WORDS

derivatization; ointment

REFERENCE
Gatti,R.; Gotti,R.; Bonazzi,D.; Cavrini,V. A comparative evaluation of three detectors in the HPLC analysis of sodium fusidate, *Farmaco*, **1996**, *51*, 115–119.

Gabapentin



Molecular formula: $C_9H_{17}NO_2$
Molecular weight: 171.24
CAS Registry No.: 60142-96-3
Merck Index: 4343

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond Elut C18 SPE cartridge with 1 mL MeOH and 1 mL buffer, do not allow to go dry. Condition an Empore C18 SPE membrane by adding 500 μL MeOH, force through three drops, discard MeOH remaining in reservoir, add 500 μL water, force through three drops, discard water remaining in reservoir. Add 200 μL 3 μg/mL IS in buffer to the SPE cartridge, add 200 μL serum and force through at 1 drop/s, add 200 μL buffer, force all liquid through, elute with 500 μL MeOH. Add 100 μL saturated sodium tetraborate solution and 50 μL 5% 2,4,6-trinitrobenzenesulfonic acid in water to the eluate, mix, heat at 50° for 10 min, add 500 μL 250 mM acetic acid, centrifuge at 12500 g for 2 min, add the supernatant to the SPE membrane, force through using a syringe or by centrifuging at 100-120 g for 5 min, wash with 500 μL MeCN:water 20:80, elute with 75 μL MeCN then 125 μL water, mix the eluates, inject a 50 μL aliquot. (Buffer was saturated sodium tetraborate solution diluted with three volumes of water.)

HPLC VARIABLES
Guard column: 20 × 2 30 μm Permaphase ETH (DuPont)
Column: 250 × 4.6 Ultrasphere C18
Mobile phase: MeCN:water:acetic acid:n-butylamine 52:48:0.1:0.01 (pH should not exceed 4.5) (Connect a 150 × 4.6 37-53 μm silica (Whatman) column between pump and injector.)
Column temperature: 50
Flow rate: 1.2
Injection volume: 50
Detector: UV 340

CHROMATOGRAM
Retention time: 10
Internal standard: 1-(aminomethyl)cycloheptaneacetic acid (13)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, caffeine, carbamazepine epoxide, carbamazepine, chlordiazepoxide, demoxepam, desalkylflurazepam, desmethylchlordiazepoxide, desmethyldiazepam, diazepam, disopyramide, ethosuximide, flurazepam, gentamicin, lidocaine, phenobarbital, phenytoin, primidone, procainamide, quinidine, theophylline, tobramycin, valproic acid, vancomycin

KEY WORDS
SPE; derivatization; pharmacokinetics

REFERENCE
Lensmeyer,G.L.; Kempf,T.; Gidal,B.E.; Wiebe,D.A. Optimized method for determination of gabapentin in serum by, *Ther.Drug Monit.*, **1995**, *17*, 251–258.

SAMPLE
Matrix: blood